

IN VITRO AND IN VIVO EFFICACY OF TR-700 AND TR-701 VERSUS THE LINEZOLID-and-METHICILLIN-RESISTANT

STAPHYLOCOCCUS AUREUS CFR STRAIN CM05

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Abstract

Background: The detection of a plasmid-borne *cfi* (chloramphenicol-florfenicol resistance) gene in staphylococci recovered from human specimens in the USA adds a new dimension to the threat against the clinical utility of several antimicrobial classes, including the oxazolidinones. A clinical strain, MRSA CM05, carries this *cfi* gene, which confers resistance to linezolid through modification of the ribosomal region involved in drug binding (A2503). TR-701 is the phosphate prodrug of the active antibacterial TR-700 currently in Phase 1 clinical trials. In this study, the activity of TR-700 and TR-701 were evaluated *in vitro* and *in vivo*, respectively, versus the linezolid resistant MRSA CM05 strain (L-MRSA).

Methods: MICs were determined by the broth microdilution method (CSLI) using a two-fold serial dilution range of test compound (64 to 0.002 µg/mL). Mouse septicemia studies were carried out using ~2x10⁷ CFU/mouse, with doses of either TR-701 or linezolid from 1 mg/kg to 50 mg/kg.

Results: MIC values obtained for TR-700 and comparator antibiotics against L-MRSA CM05 were as follows: TR-700 = 0.5 µg/mL, linezolid = 8 µg/mL, daptomycin = 2 µg/mL, oxacillin >64 µg/mL; vancomycin = 1 µg/mL, trimethoprim = 1 µg/mL, ciprofloxacin = 16 µg/mL, and tetracycline = 0.125 µg/mL. A single oral dose of 20 mg/kg TR-701 provided 100% protection in animals 24 hours post-infection. In contrast, only 1 of 10 animals survived when treated with linezolid at 20 mg/kg.

Conclusions: TR-700 was 16-fold more potent than linezolid against CM05, with ciprofloxacin and oxacillin having little or no antimicrobial activity against the isolate. In the mouse septicemia model, TR-701 was effective in protecting mice systemically infected with the L-MRSA CM05 strain, whereas linezolid provided only marginal protection at the highest concentration evaluated (50mg/kg).

Materials and Methods

In Vivo Efficacy in Mouse Septicemia Model:

Ninety DBA/2 female mice, 6-7 weeks old, were randomized to 1 of the 9 treatment groups listed in Table 1.

Cyclophosphamide Treatment: On Day -4 pre-infection, all mice received 150 mg/kg cyclophosphamide by IP injection. On Day -1 mice received 100 mg/kg cyclophosphamide by IP injection. Animal weights were obtained immediately prior to each cyclophosphamide treatment.

Bacterial Inoculation: Day 0, all animals were infected with L-MRSA CM05 by injecting 100 µL (~2x10⁷ cfu/mouse) IP. (Table 2)

Drug Administration Dosing: TR-701 or linezolid was administered immediately post infection via oral gavage administration. Doses were 50, 20, 10, 5, or 1 mg/kg based on average weight of all the mice.

Survival: At 24 hours post-infection, the number of surviving mice was determined. Surviving mice were clinically evaluated, then euthanized. Kidneys were surgically removed from the highest dosed groups of surviving mice, placed into tubes and frozen immediately. Collected kidneys were homogenized in 900 µL sterile water. 10-fold dilutions were made into sterile water. 50 µL of each dilution was spotted onto 5% blood agar plates. Plates were allowed to dry then incubated at 37°C for 20 hours. Well isolated colonies were chosen for susceptibility testing.

Results

Table 1. Treatment Groups for Mouse Septicemia Efficacy Study

Group	Treatment	Oral Dose mg/kg
A1	Untreated	-
B1	TR-701	1
B2	TR-701	5
B3	TR-701	10
B4	TR-701	20
C1	Linezolid	5
C2	Linezolid	10
C3	Linezolid	20
C4	Linezolid	50

Table 2. Procedure for MRSA CM05 Mouse Septicemia Efficacy Study

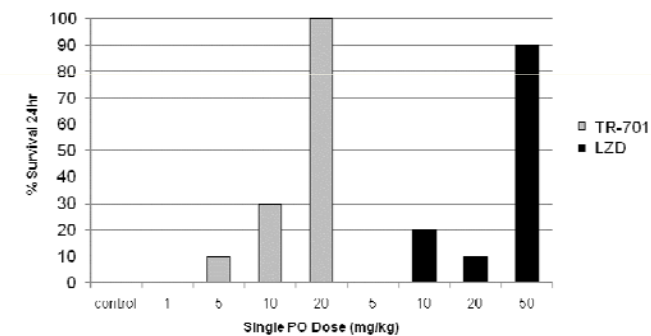
Day	Treatment	Amount/Timing
Day -4	Cyclophosphamide - IP injection	150 mg/kg
Day -1	Cyclophosphamide - IP injection	100 mg/kg
Day 0	Infection - 100 µL MRSA CM05 suspension, IP injection	2.2 x 10 ⁷ CFUs/mL
	Drug administration - oral gavage	Immediately post-infection
Day 1	Score for survival Record clinical signs Recover infected kidneys Determine CFUs Perform MIC evaluation	

Table 3. Summary of Clinical Signs for MRSA CM05 Infected Animals 24 Hours Post-Treatment with TR-701 or Linezolid

Group	Treatment	Dose mg/kg	Survival	Clinical Score (No.) ^a
A1	Untreated	-	0/10	-
B1	TR-701	1	0/10	-
B2	TR-701	5	1/9	-
B3	TR-701	10	3/10	++ (2), +++ (1)
B4	TR-701	20	10/10	++ (8), +++ (2)
C1	Linezolid	5	0/10	-
C2	Linezolid	10	2/10	++ (2)
C3	Linezolid	20	1/10	+++ (1)
C4	Linezolid	50	9/10	+++ (9)

^a "No." corresponds to the number of animals presenting clinical signs in a given category.
Clinical Observation Scoring:
() No symptoms (+) moderate signs of sickness: decreased activity, ruffled hair
(++) very sick: labored breathing, hunched, noticeable weight loss, decreased activity
(+++) non-responsive, moribund

Figure 1. TR-701 Demonstrates Greater Efficacy Than Linezolid in CM05 Infected Mice, 24 Hrs. Post Infection



Animals received a single oral dose of either TR-701 or linezolid (LZD) at the doses indicated, immediately post-infection. The percentages of animals surviving 24 hours post-treatment are plotted.

Table 4. MIC Values for MRSA CM05 Isolates and Control *S. aureus* Strains versus TR-700 and 7 Comparator Drugs

Antibiotic	MRSA CM05 Inoculum	MIC µg/mL			
		MRSA CM05 Isolates from Treated Animals		MSSA ATCC13709	MRSA ATCC33591
		TR-701 Treated	Linezolid Treated		
TR-700	0.5	0.5	0.5	0.5	0.5
Linezolid	8	8	8	2	2
Tetracycline	0.125	0.125	0.125	0.25	0.25
Ciprofloxacin	16	16	16	0.25	0.5
Trimethoprim	1	1	1	1	1
Vancomycin	1	1	1	1	1
Daptomycin	2	2	2	0.5	2
Oxacillin	>64	>64	>64	0.5	>64

Presented here are the MIC values for strains isolated from animals infected with MRSA CM05 and treated with either TR-701 or linezolid (Table 3). The results demonstrate retention of the initial antibiotic susceptibility profile by the recovered organisms.

Materials and Methods cont.

MIC Determinations:

MICs were determined by broth microdilution according to Clinical and Laboratory Standards Institute method M7-A7.¹ and interpreted using Alamar Blue to visualize cell viability/cell killing.² Assays were conducted in Mueller Hinton-cationic adjusted medium over a two-fold serial dilution range from 64 to 0.002 µg/mL of test compounds. The strains tested were *S. aureus* MRSA CM05 (linezolid-resistant strain)³, *S. aureus* MRSA (ATCC 33591) and *S. aureus* MSSA (ATCC 13709). Isolated colonies obtained from infected kidneys were tested in parallel for comparison.

Results and Discussion

A mouse septicemia model was developed to evaluate the *in vivo* activity of TR-700 against MRSA CM05 when administered as the prodrug TR-701.

- Mice were infected with MRSA CM05 then treated with a single oral dose of either TR-701 or linezolid. 24 hours post-infection, animals were scored for survival and clinical signs recorded (Table 3).
- All 10 untreated animals (control) died by 24 hours post-infection. In contrast, TR-701 treatment protected mice in a dose-dependent manner, with 20 mg/kg TR-701 providing complete protection (i.e., 100% survival). Linezolid treatment was effective only at the highest concentration tested (50 mg/kg), with incomplete protection. Surviving animals in this treatment group, however, appeared very sick to non-responsive or moribund, whereas the majority of mice administered TR-701 at the highest dose displayed milder clinical signs, such as ruffled hair or decreased activity. (Figure 1)
- The antimicrobial profile for the MRSA CM05 isolate used for efficacy studies was determined with TR-700, linezolid and 6 additional antibiotics (Table 4). This study demonstrated a range of drug sensitivities, with the organism highly susceptible to TR-700 (MIC = 0.5 µg/mL). TR-700 was 2- to > 32-fold more potent *in vitro* than all tested antibiotics with the exception of tetracycline (MIC = 0.125 µg/mL). Linezolid was 16-fold less active than TR-700, with an MIC value = 8 µg/mL.

Conclusions

- Oral TR-701, the prodrug of TR-700, was effective in protecting mice systemically infected with this strain, whereas linezolid provided only marginal protection at the highest concentration evaluated.
- The linezolid non-susceptible *S. aureus* strain *cfi* CM05 was highly susceptible to inhibition by TR-700 *in vitro*.
- TR-701 has the therapeutic potential to treat infections due to linezolid-resistant strains including strains harboring the transposon/plasmid-borne *cfi* resistance gene.

Reference

CLSI 2006, Document M7-A7
Barrow *et al.* 2006, Antimicrob. Agents Chemother. 27: 178-180
Toh *et al.* 2007, Mol. Microbiol. 64:1506-1514